

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL
SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> PCA40739/HMY

<150> KR1020030072216

<151> 2003-10-16

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1

gggaagcttc gatcggacat ccagatgacc cagtcctccat cctccctgtc tgcattctgta 60

ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 2

tggtttttgc tgataccagg ctaagtaatt tctgatgcc tgacttgccc gacaagtgat 60

ggtgactctg tcccctacag

80

<210> 3
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 3
cctggatatca gcaaaaacca gggaaagccc ctaagctcct gatctatgct gcatccactt 60
tgcaatcagg ggtoecatct 80

<210> 4
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 4
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agatgggacc cctgattgca 80

<210> 5
<211> 80
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<220>
<223> gene fragment of light chain variable region

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<400> 5
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caccgtatac ttttggccag 80

<210> 6
<211> 41
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<400> 6
tttgatttcc accttgggtcc cctggccaaa agtatacggt g 41

<210> 7
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 7
gggaagcttc gatcggaggt gcagctggtg gagtctgggg gaggcttggg acagcccggc 60
aggtccctga gactc 75

<210> 8
<211> 79
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

4

<400> 8
agcttgccgg acccagtgc tggcataatc atcaaagggtg aatccagagg ccgcacagga 60
gagtctcagg gacctgccg 79

<210> 9
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 9
tgcactgggt ccggcaagct ccaggggaagg gcctggaatg ggtctcagct atcacttga 60
atagtgggtca catagactat 80

<210> 10
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 10
atacagggag ttcttggcgt tgtctctgga gatggtgaat cggccctcca cagagtccgc 60
atagtctatg tgaccactat 80

<210> 11
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 11
acgccaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60
attactgtgc gaaagtctcg 80

<210> 12
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 12
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aaggaggagac gcggtgctaa 60
ggtacgagac tttcgcacag taat 84

<210> 13
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for heavy chain

<400> 13
cccaagctta ggcctccacc aagggcccat cggctcttc 39

<210> 14
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for heavy chain

<400> 14
gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for light chain

<400> 15
cccaagcttt cgcgaaactgt ggctgcacca tctgtcttca tc 42

<210> 16
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for light chain

<400> 16
cccggatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17
<211> 69
<212> DNA
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17
atgaaaaaga caatcgcat tcttcttgca tctatgttcg ttttttctat tgctacaaat 60

gcccaggcg 69

7

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing *Stu*I restriction enzyme site

<400> 18
tctattgcta caaatgccca ggccttccca accattccct tatcc 45

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing *Stu*I restriction enzyme site

<400> 19
agataacgat gttacgggt ccggaagggt tggtaaggga atagg 45

<210> 20
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer specific for light chain

<400> 20
gggggatcct caccgggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 21
<211> 43
<212> DNA
<213> Artificial Sequence

<220>

<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21

gggggatcca ggaggtgatt tatgaaaag acaatcgcat ttc

43

<210> 22

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> forward primer containing BpuI restriction enzyme site

<400> 22

ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttic

44

<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer containing BpuI restriction enzyme site

<400> 23

ggggctcagc tcacgcggcg catgtgtgag tttgtcaca agatttaggc tc

52

<210> 24

<211> 63

<212> DNA

<213> E. coli OmpA signal sequence

<400> 24

atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa

60

gct

63

<210> 25

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> forward primer specific for heavy chain

<400> 25

gaggttcagc tagtcgagtc aggaggcggt

30

<210> 26

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> forward primer containing HindIII and StuI restriction enzyme sites

<400> 26

gggagatctt cagcgggcgc atgtgtgagt ttgtcaca gatttaggct c

51

<210> 27

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 27

gacattcaaa tgaccagag cccatccagc

30

<210> 28

10

<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28
cccagatctc taacactctc cctgttgaa gctctttgtg ac 42

<210> 29
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29
ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing SalI restriction enzyme site

<400> 30
ggggtcgact cgcgcggcgc atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 31
<211> 42
<212> DNA
<213> Artificial Sequence

11

<220>

<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31

gggcataatga aaaagacaat cgcatitctt cttgcatcta tg

42

<210> 32

<211> 705

<212> DNA

<213> Artificial Sequence

<220>

<223> TNF-alpha heavy chain

<400> 32

gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc	60
tcctgtgcgg cctctggatt cacctttgat gattatgccatgca tgcactgggt ccggcaagct	120
ccaggaagg gcctggaatg ggtctcagct atcacttggga atagtgggtca catagactat	180
gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat	240
ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg	300
taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggg caccgtctcg	360
agtcctcca ccaagggccc atcgggtctc cccctggcac cctcctccaa gacacacctt	420
gggggcacag cggccctggg ctgcctgggc aaggactact tccccgaacc ggtgacggg	480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tccgggtgt cctacagtcc	540
tcaggactct actccctcag cagcgtggg accgtgccct ccagcagctt gggcaccag	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc catag	705

12

<210> 33
 <211> 645
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TNF-alpha light chain

<400> 33
 gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc 60
 atcacttgtc gggcaagtca gggcatcaga aattacttag cctggatatca gcaaaaacca 120
 gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct 180
 cggttcagtg gcagtggatc tgggacagat ttactctca ccatcagcag cctacagcct 240
 gaagatgttg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300
 gggaccaagg tggaatcaa acgaactgtg gctgcacat ctgtcttcat ctccccgcca 360
 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag 480
 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34
 <211> 7
 <212> PRT
 <213> TNF-alpha light chain

<400> 34
 Asp Ile Gln Met Thr Gln Ser
 1 5

13

<210> 35
<211> 8
<212> PRT
<213> TNF-alpha heavy chain

<400> 35
Glu Val Gln Leu Glu Val Asp Ser
1 5

<210> 36
<211> 12
<212> PRT
<213> N-terminal sequence of recombinant TNF-alpha

<400> 36
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
1 5 10